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Sex and the Single Splice

Drosophila **male courtship offers a unique experimental system to uncover the molecular and neural basis of genetically preprogrammed behavior. In this issue of *Cell*, Demir and Dickson (2005) and Stockinger et al. (2005) demonstrate that this behavior relies on a single splicing of the *fruitless* transcript, and on a specific olfactory-based neuronal circuit.**

The mechanisms by which complex behaviors are orchestrated within the brain, and that direct the establishment of corresponding neural circuits, remain one of the great mysteries of neuroscience. On the one hand, the amazing ability of animals to learn and to adapt their behavior to the external environment points to the existence of a large range of neural plasticity in behavioral networks. On the other hand, elaborate courtship behaviors, complex nest building in birds, and spider web weaving constitute striking examples of stereotyped and species-specific behavioral sequences that do not require any previous sensory or behavioral experience of the individual nor any contact with members of the species. These fixed patterns of behavior argue against the learned nature of all behaviors and suggest instead the existence of genetically transmittable organizers of behaviors. Inspired by the success of forward genetics in identifying developmental regulators of complex morphological structures such as the animal wings, legs, or body plan, some authors have predicted that single or few genes could act during brain development as master regulators of behavior (Benzer 1973; Baker et al., 2001). In favor of this hypothesis, mutations in transcriptional regulators have been identified that cause remarkably specific behavioral defects both in vertebrates and invertebrates: for example, the *fruitless* mutation in *Drosophila* impairs all steps of male courtship and sex discrimination (reviewed in Baker et al., 2001), whereas in mice, *fosB* mutant displays abnormal maternal behavior (Brown et al., 1996), and mutation in the *Hoxb8* gene affects mouse grooming behavior (Greer and Capecchi, 2002). However, only rare mutants exhibiting exclusive and specific behavioral defects and no associated morphological defects have ever been identified, making it difficult to strongly support a general model according to which "behavioral master gene regulators" would work independently from "developmental master gene regulators." Moreover it was noted that most of the transcription factors affected in behavioral mutants have broad distributions and general functional properties that seem incompatible with a role as genetic determinants of specific behaviors. These observations have suggested that instead of dedicated behavioral organizers, one should instead consider the additive effect and evolution of a myriad of transcription regulators and of other neuronal genes that all together contribute to the development of specific behavioral networks. In further support of this alternative model, an increasingly large cohort of mutations and polymorphisms in genes involved in neuronal signaling has been identified that generate remarkably specific behavioral variations (de Bono and Bargmann, 1998; Stowers et al., 2002; Leypold et al., 2002).

The debate on this issue is far from closed: two articles, in this issue of *Cell*, from the laboratory of Barry Dickson provide new ammunition to the proponents of simple behavioral organizers by demonstrating the causal relationship between a single splicing event in the *fruitless* gene and the emergence of male courtship behavior in *Drosophila* and by providing insight into the nature of the neural circuit underlying this behavior.

From Transcript Splicing to Complex Behavior

The *Drosophila fruitless* (*fru*) mutant has long intrigued behavioral geneticists because of its remarkable behavioral specificity: male *fru* are affected in every step of the stereotyped fly courtship behavior and will mate indiscriminately with both males and females. The behavioral defect of *fru* mutants is often represented by the *fru* male mating chains, where mutant males are both trying to mate and are mated with. *Fru* females do not display any behavioral phenotype, and the only nonbehavioral phenotype in the male is a defect in the formation of a male-specific muscle called the muscle of Lawrence. Interestingly, identification of the *fru* gene (reviewed in Baker et al., 2001) has shown that, through the use of multiple promoters and splicing variants, *fru* encodes a set of potential transcription factors containing a BTB domain and zinc-finger motifs. It appears that *fru* loss of function is lethal because transcripts generated in the 3′ portion of the gene play essential sex-nonspecific functions such as imaginal disk formation and synaptogenesis (Figure 1). In contrast, the sex and behavioral specificity of the *fru* mutations has been mapped to the 5′ end of the gene: sex-specific transcripts are generated from the upstream P1 promoter, and males and females are likely to synthesize proteins that differ in their N terminus from a unique differential splicing event (Figure 1). More specifically, a female-specific exon introduces a stop codon 3′ of the first Methionine. Thus females can only synthesize a 3′- or 5′-truncated protein and, in fact, are most likely to make no protein at all (Usui-Aoki et al., 2000).

How does *fru* control male-specific reproductive behavior? In this issue of *Cell*, Demir and Dickson (2005)

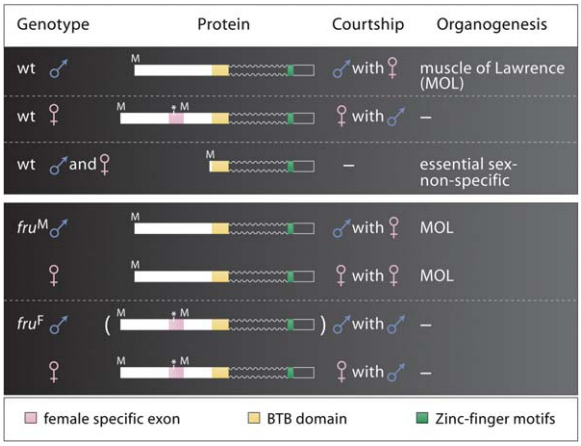

Figure 1. Sex-Specific FRU Proteins Control Courtship Behavior and Sex Discrimination in *Drosophila*

The *fruitless* gene is transcribed from multiple promoters and displays a vast range of differential splicing events. Sex-specific transcripts are generated from a 5′-located promoter, and a single sexually dimorphic splicing event generates the male (FRU^M) or the female (FRU^F) specific protein. Forced expression of the sex-specific transcripts in males or females shows that FRU^M is necessary and sufficient to induce male courtship and the formation of the muscle of Lawrence, whereas FRU^F abolishes male courtship. Parentheses around FRU^F in males indicates that the female-specific splicing may be limited by lack of the appropriate splicing factors in males.

provide a direct demonstration of the necessary and essential role of a single splicing event in *fru* to control the emergence of male courtship with females. Using homologous recombination in *Drosophila* to force transcription of specific splicing variants in fly of either sex, they show that males require the expression of the male form of the protein (FRU^M, Figure 1) in order to display courtship toward females and that expression of the female protein in male (FRU^F, Figure 1) abolishes courtship. Furthermore, females forced to express FRU^M instead of FRU^F lose all female-specific behavioral traits (egg laying and reproduction with males) and acquire instead a striking male behavior: although they are morphologically females, they display the full sequence of the male-like courtship steps when placed with other females. From these studies, one can firmly argue in favor of *fru* acting through a single splicing event as the sufficient and necessary master organizer of fly male courtship.

From Single Splicing to Behavioral Circuit

How can a single splicing event specify a complex sequence of stereotyped behaviors? It has been shown that expression of *fru* at the pupal stage is essential for the correct display of adult male behavior. Moreover, *fru* encodes a set of putative zinc-finger transcription factors, and the male-specific protein FRU^M has been identified in distinct neuronal clusters of the male brain, some of which are known to play a role in specific steps of the male courtship. It has therefore been postulated that *fru* could act as a behavioral organizer specifying the neuronal network essential for male courtship. Using an elegant series of genetic manipulations that

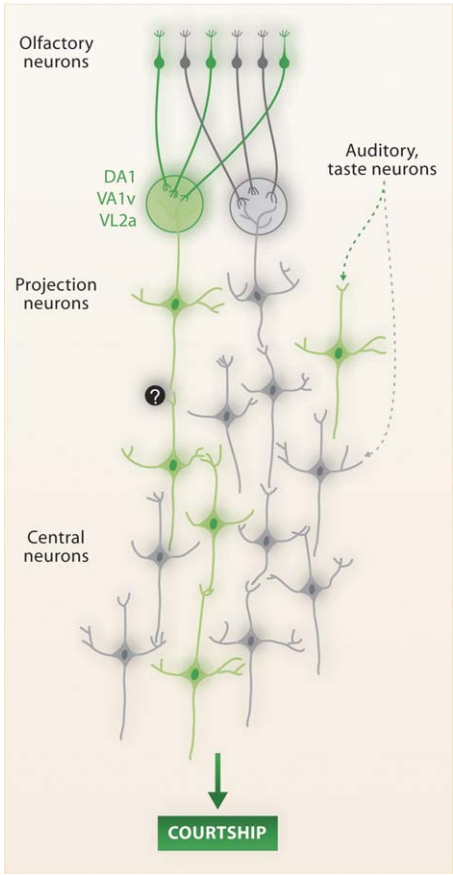

Figure 2. Fru-Expressing Neurons, Including Specific Olfactory Neurons Form the Neural Circuit Controling Courtship Behavior and Sex Discrimination

Expression of a reporter gene in neurons expressing FRU^M led to the anatomical and functional characterization of the *fru* circuit. A specific subset of olfactory neurons sending projections to the sexually dimorphic glomeruli DA1, VA1v, and VL2a and connected to fru-positive projection neurons of the antennal lobe are essential components of the courtship behavioral circuit. Other sensory neurons in auditory and gustatory structures, as well as neurons throughout the brain, express fru and may form a complex circuit orchestrating the male courtship behavior. Surprisingly this circuit appears also to exist in females.

enabled them to visualize and manipulate neurons expressing the sex-specific *fru* transcripts, Stockinger et al. (2005) have performed the anatomical and functional dissection of the neuronal circuit driving male courtship. In addition to the already known FRU^M-positive neuronal clusters in the brain, Stockinger et al. were able to identify a subset of FRU^M-positive olfactory-, auditory-, and taste-sensory neurons, all belonging to sensory modalities known to play a role in male courtship. Remarkably, FRU^M-positive olfactory-sensory neurons appear exclusively connected to all known sexually dimorphic glomeruli in the fly antennal lobe, which in turn receive dendritic input from FRU^M-positive projection neurons of the antennal lobe (Figure 2). Moreover, the silencing of FRU^M-positive olfactory-sensory neurons appears to reduce courtship to the same extent as the silencing of all FRU^M-positive brain neurons.

These data beautifully demonstrate that a specific subset of olfactory neurons, presumably detecting pheromones, are essential triggers of the male courtship behavior, and that they are functionally connected to other FRU^M-positive neurons in the brain. Surprisingly, however, the same circuit can be identified both in the male and the female brain, with only minor differences that are hard to reconcile with the striking sexual dimorphism of the fly reproductive behavior. Thus, the mystery of *fru* function as a behavioral organizer appears to grow even deeper. Hopefully, however, the further characterization of the FRU^M circuit will provide a unique opportunity to uncover the developmental and neuronal mechanisms underlying the control of instinctive behaviors.

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